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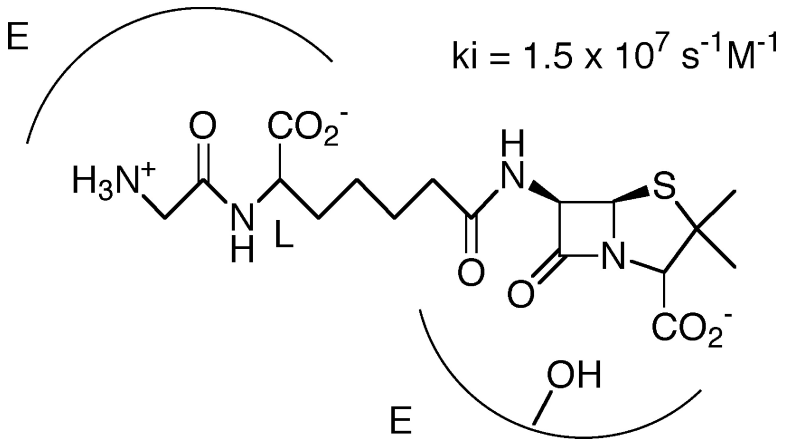

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The Perfect Penicillin? Inhibition of a Bacterial DD-Peptidase by Peptidoglycan-Mimetic β-Lactams

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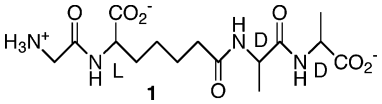
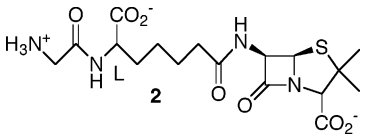
The inactivation of bacterial DD-peptidases by β-lactams is the reaction responsible for the antibiotic action of the latter molecules.[1,2] Although a large number of β-lactams have been tested for antibiotic activity and, in general, the most effective of them have been chosen for clinical application, the relationship between the β-lactam structure and antibiotic activity is not generally predictable because of the large number of contributing factors. Even the structural basis for the specificity of the β-lactam for the target DD-peptidase is not well understood. One might imagine, following the proposal of Tipper and Strominger,[2] that good peptide substrates of DD-peptidases and good β-lactam antibiotics would have side-chains resembling those of the stem peptides of bacterial peptidoglycan biosynthesis. There is, however, to date, little direct evidence in favor of this idea and, curiously, a number of indications to the contrary.[3,4]

Recently, research in this laboratory has demonstrated that peptide **1** is an excellent substrate of the *Streptomyces* R61 DD-peptidase and the best peptide substrate yet discovered for a DD-peptidase, with a k_{cat}/K_m value of 8.7×10^6 s^{-1} M^{-1}.[5]



1

This peptide is distinguished by the presence of an amine terminus characteristic of the stem peptide of *Streptomyces* sp. Although studies with less specific substrates of this enzyme have suggested that side-chain specificity may be different in peptide substrates than in β-lactam inhibitors,[3,6−9] the question of the relative reactivity of peptides and β-lactams that contain highly specific side-chains has not yet been directly addressed. In this paper, we describe the synthesis of the penicillin **2** and the cephalosporin **3** (Supporting Information), each bearing the *Streptomyces*-specific side chain present in **1**.



2

The data presented below show that **2** is an excellent inhibitor of the R61 DD-peptidase, with a second-order inactivation rate constant of 1.5×10^7 s^{-1} M^{-1}. This represents, to our knowledge, the largest rate constant yet reported for inactivation of a DD-peptidase by a β-lactam and directly addresses the question referred to above.

Compounds **2** and **3** were found to rapidly inactivate the *Streptomyces* R61 DD-peptidase. From assays of mixtures of the enzyme with increasing concentrations of **2**, for example, the titration curve shown in Figure 1 was obtained. This indicates that

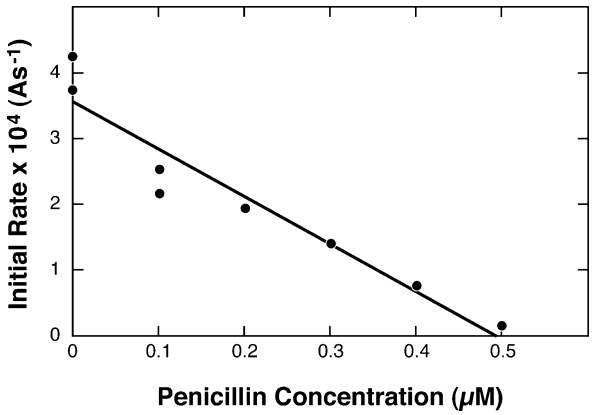
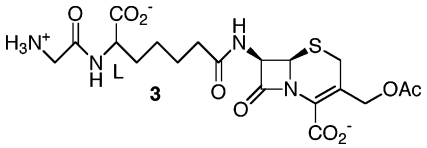


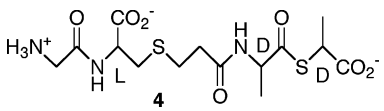
Figure 1. Titration of **2** against the R61 DD-peptidase. The graph shows residual activity against **1** after incubation of the enzyme (0.4 μM) with **2** (0−0.5 μM).

the DD-peptidase is inactivated by **2** in a stoichiometric 1:1 fashion, as anticipated for the reaction of a β-lactam with a DD-peptidase.



3

Measurement of the second-order rate constant for the inactivation, k_i, could not be made directly by manual mixing methods or by competition with classical β-lactams such as cephalothin because the reaction was too rapid. Finally, measurement of the rate constant with good precision, was found to be possible from competition experiments employing the specific chromophoric thiolester **4** as the substrate ($k_{cat} = 42$ s^{-1}, $K_m = 4.0$ μM, $k_{cat}/K_m = 1.05 \times 10^7$ s^{-1} M^{-1}).



4

Figure 2A shows spectrophotometric total progress curves for reaction of the DD-peptidase with **4** in the absence and presence of **2**; in the latter case, **2** inactivates the enzyme prior to complete turnover of **4**. These curves, taken from a series of experiments at several concentrations of **2** and **4**, could then be fitted to Scheme 1 by the Dynafit program[10] to yield a k_i value of $(1.0 \pm 0.2) \times 10^7$ s^{-1} M^{-1}. The rate constant for inactivation could also be obtained fluorimetrically[11] in a stopped flow experiment. Figure 2B shows the result of such an experiment. A series of such runs gave a value of k_i of $(2.0 \pm 0.4) \times 10^7$ s^{-1} M^{-1}, in good agreement with the value obtained from the competition experiment described above.

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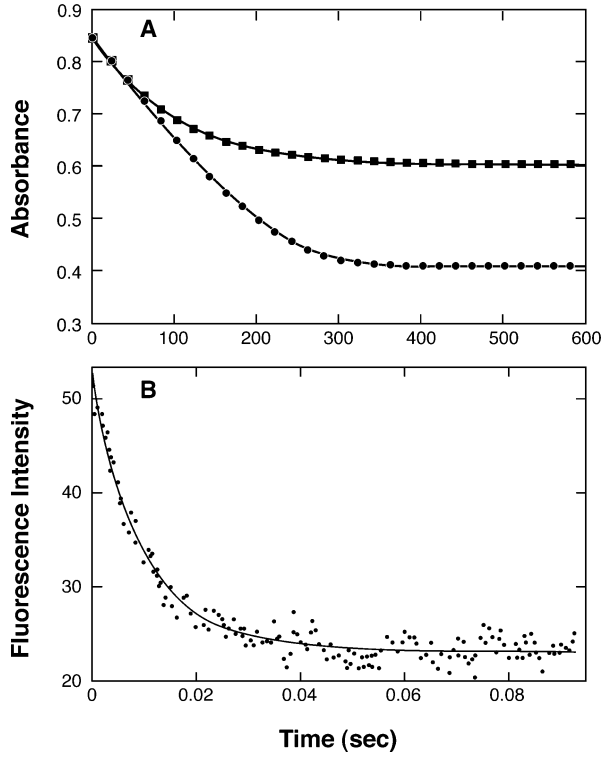

Figure 2. (A) Total progress curve for hydrolysis of the substrate **4** (300 μM) by the R61 DD-peptidase (30 nM) in the absence (filled circles) and presence (filled squares) of **2** (40 nM). The reaction was monitored spectrophotometrically at 240 nm. (B) Total progress curve for direct reaction of **2** (6 μM) with the R61 DD-peptidase (1.67 μM). The reaction was monitored fluorimetrically at 324 nm.

No indication was observed of enzyme saturation by the inhibitor at the concentrations employed ($\leq$6.0 μM).

Scheme 1

$$E + S \underset{Km}{\rightleftharpoons} ES \xrightarrow{kcat} E + P$$

$$E + I \xrightarrow{ki} E\text{-}I$$

The rate constant for inactivation of the enzyme by the cephalosporin **3** was also determined from competition experiments with the substrate **4**; the value of k_i thus obtained was $(5.6 \pm 1.5) \times 10^5\ s^{-1}\ M^{-1}$. The R61 DD-peptidase is usually more susceptible to penicillins than to cephalosporins.[6]

Although **2** reacts rapidly with the R61 DD-peptidase, it is not a good substrate of the class C *Enterobacter cloacae* P99 β-lactamase despite the structural similarity between these two enzymes.[12] Values of the steady-state parameters for hydrolysis (the reaction was confirmed by an NMR experiment) of **2** by this enzyme were $k_{cat} = (1.0 \pm 0.2)\ s^{-1}$, $K_m = (0.48 \pm 0.21)$ mM, and $k_{cat}/K_m = 2.1 \times 10^3\ s^{-1}\ M^{-1}$. These values can be contrasted with those of benzylpenicillin, viz. $k_{cat} = 50\ s^{-1}$, $K_m = 1.5\ \mu$M, and $k_{cat}/K_m = 3.3 \times 10^6\ s^{-1}\ M^{-1}$. It appears that, as has been frequently suggested,[13] and as Meroueh et al.[14] also noted recently, β-lactamases have evolved to exclude peptides and, particularly, extended sections of peptidoglycan, from their active sites. Although this seems reasonable, given the side chain structures of classical β-lactams, it is not clear, as reinforced by the present results, why β-lactams with peptidoglycan-mimetic side-chains have not been selected for interbacterial warfare. Perhaps they proved to be too dangerous to handle? Or is there an elegant defense?

The results described above show that **2** and **3** are extremely potent inactivators of the *Streptomyces* R61 DD-peptidase. For perspective, benzylpenicillin, the hitherto reported most effective β-lactam inhibitor of this enzyme, reacts with a rate constant of $1.37 \times 10^4\ s^{-1}\ M^{-1}$ and the cephalosporin cephalothin with a rate constant of $1.4 \times 10^3\ s^{-1}\ M^{-1}$.[6] Thus, it is clear that, at least in the case of this DD-peptidase, a peptidoglycan-mimetic side chain dramatically improves the effectiveness of a β-lactam inhibitor as it does a peptide substrate.[5] It is possible that the reactions of both **1** and **2** with the enzyme are diffusion-limited. It was not possible to readily test this proposition by a direct viscosity experiment since it appeared that both glycerol and sucrose significantly depressed the reactivity of the enzyme against both benzylpenicillin and cephalothin; these latter reactions cannot be diffusion-controlled.

It should be noted that β-lactams with peptide and peptidoglycan-mimetic side-chains have previously been reported in the literature.[15,16] In general, they have been found to not be good substrates. There are, as noted above, many reasons why this might be so, but in no case to date has their reactivity with a specific target enzyme been studied. On the other hand, there is evidence that nonspecific peptide side-chains on β-lactams yield poor DD-peptidase inhibitors.[17] At any event, there is now one unambiguous case where incorporation of a specific peptidoglycan-mimetic side chain into a β-lactam has had a dramatic positive effect on its reactivity with a DD-peptidase. It will be of interest to explore the generality of this result and to consider its implications to the further design of DD-peptidase inhibitors.

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Supporting Information Available: Synthetic procedures for compounds **2** and **3**, as well as kinetics methods (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Martin, H. H. J. *Gen. Microbiol.* **1964**, *36*, 441.
(2) Tipper, D. J.; Strominger, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 1133.
(3) Xu, Y.; Soto, G,; Adachi, H.; Van der Linden, M. P. G.; Keck, W.; Pratt, R. F. *Biochem. J.* **1994**, *302*, 851.
(4) Anderson, J. W.; Adediran, S. A.; Charlier, P,; Nguyen-Distèche, M.; Frère, J.-M.; Nicholas, R. A.; Pratt, R. F. *Biochem. J.* **2003**, *373*, 949.
(5) Anderson, J. W.; Pratt, R. F. *Biochemistry* **2000**, *39*, 12200.
(6) Ghuysen, J.-M.; Frère, J.-M.; Leyh-Bouille, M.; Coyette, J.; Dusart, J.; Nguyen-Distèche, M. *Annu. Rev. Biochem.* **1979**, *48*, 73.
(7) Lammotte-Brasseur, J.; Dive, G.; Ghuysen, J.-M. Eur. *J. Med. Chem.* **1984**, *19*, 319.
(8) Lamotte, J.; Dive, G.; Ghuysen, J.-M. *Eur. J. Med. Chem.* **1991**, *26*, 43.
(9) Neuhaus, F. C.; Georgopapadakou, N. In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*; Sutcliffe, J., Georgopapadakou, N., Eds.; Chapman and Hill, New York: 1991; p 205.
(10) Kuzmic, P. *Anal. Biochem.* **1996**, *237*, 2602.
(11) Jamin, M.; Adam, M.; Damblon, C.; Christiaens, L.; Frère, J.-M. *Biochem. J.* **1991**, *280*, 499.
(12) Knox, J. R.; Moews, P. C.; Frère, J.-M. *Chem. Biol.* **1996**, *3*, 937.
(13) Pratt, R. F. *J. Chem. Soc., Perkin Trans. 2* **2002**, 851.
(14) Meroueh, S. O.; Minasov, G.; Lee, W.; Shoichet, B. K.; Mobashery, S. *J. Am. Chem. Soc.* **2003**, *125*, 9612.
(15) Bentley, P. H.; Stachulski, A. V. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1187.
(16) Hanessian, S.; Couture, C. A.; Georgopapadakou, N. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2323.
(17) Lee, M.; Hesek, D.; Suvorov, M.; Lee, W.; Vakulenko, S.; Mobashery, S. *J. Am. Chem. Soc.* **2003**, *125*, 16322.

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